Eimeria telekii n.sp. (Apicomplexa: Coccidia) from *Lemniscomys striatus* (Rodentia: Muridae): morphology, pathology and phylogeny

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SUMMARY

Using a combination of morphological, life-cycle and molecular data, we describe a new apicomplexan parasite *Eimeria telekii* n.sp. from a striped grass mouse *Lemniscomys striatus* captured in Kenya. Oocysts are oval to spherical or ellipsoidal, 20·4 × 15·7 (15·5–25·0 × 12·0–20·0) μm with a colourless, smooth and bilayered wall. Sporocysts are ellipsoidal, 11·2 × 7·8 (10·0–12·0 × 7·0–9·0) μm with a small Stieda body and granular sporocyst residuum and contain 2 elongated, banana-shaped sporozoites with a single refractile body. Life-cycle, pathogenicity and host specificity of this parasite were studied in laboratory-bred *Lemniscomys barbarus* and BALB/c mice. Two asexual stages and the sexual phase took place within the enterocytes of the caecum and colon of *L. barbarus* but not in inoculated BALB/c mice. An infectious dose of 5000 oocysts caused severe clinical illness and mortality in 2/2 (100%) *L. barbarus*. Phylogenetic analysis of the small subunit rRNA gene of *E. telekii* and members of the genera *Eimeria*, *Cyclospora* and *Isospora* placed *E. telekii* within the eimerian rodent clade.†

Key words: *Eimeria telekii* n.sp., coccidia, life-cycle, pathology, genetic analysis, SSU rRNA.

INTRODUCTION

The coccidia comprise a large group of obligate parasitic protozoa (Pellérdy, 1974). Several members of the most species-rich genus *Eimeria* cause considerable morbidity and mortality in livestock and wildlife and are thus pathogens of medical and veterinary importance (Long, 1990). Species identification of coccidia is traditionally based on host specificity, site of infection, and morphology of oocysts and other life-cycle stages. These features, however, sometimes overlap among species and may vary within a single species (Gardner & Duszynski, 1990; Parker & Duszynski, 1986; Upton *et al*. 1992). Therefore, the importance of molecular characterization for species identification and systematics of these parasites is growing (Cere, Licois & Humbert, 1995; Hnida & Duszynski, 1999*b*; Johnson & Fernando, 1995). Because of good resolution at the generic and specific levels, the small subunit (SSU) ribosomal RNA gene is a molecular marker of choice for the studies of evolutionary relationships among apicomplexan parasites (Barta *et al*. 1997; Carreno & Barta, 1999; Lopez *et al*. 1999). It is desirable that new descriptions are based on a combination of morphological and molecular approaches.

Eimerian infections are widespread among rodents (Levine & Ivens, 1965). However, very limited data on the coccidia of rodents from sub-Saharan Africa are available. So far, only 2 eimerian species have been described from mice of the genus *Lemniscomys*: *Eimeria lemniscomysis* and *E. putevelata*, both from Liberia (Bray, 1958; Levine *et al*. 1959). In the present study we describe a coccidium from *L. striatus*, an abundant murid species inhabiting almost all of sub-Saharan Africa. Life-cycle, endogenous development, pathogenicity and host specificity of this parasite were studied in laboratory-bred *L. barbarus* and in BALB/c mice. On the basis of morphological, experimental and sequence data we conclude that this isolate is a new species as described below.

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† The nucleotide sequence was deposited in the GenBank™ under the accession number AF246717.

MATERIALS AND METHODS

Wild-caught animals

Lemniscomys striatus (Linnaeus, 1758) were trapped in a flower garden in Chuka (1550 m) near Mt Kenya

Forest Reserve, Kenya, during a field study in September 1998 and in October 1999. Mice ($N = 4$) were necropsied and the ethanol-fixed symbiotype was deposited at the mammalian section of the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany (ZFMK). The intestinal contents were placed in 2·5 % (w/v) potassium dichromate solution ($K_2Cr_2O_7$), and the tissue samples of the gastrointestinal tract were fixed in 10 % buffered formalin. For a follow-up examination the samples were shipped to the University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

The intestinal contents were screened for coccidia using flotation in Sheather's sugar solution (specific gravity 1·30). Coccidian oocysts were examined and measured with a calibrated ocular micrometer using bright-field microscopy and photographed using an Olympus BX 60 microscope equipped for Nomarski interference contrast microscopy. Measurements are in μm as the mean followed in parentheses by the range and number (N) of stages measured.

Experimental inoculation

For experimental inoculations 11 adult, laboratory-reared, coccidia-free *L. barbarus* and 6 coccidia-free BALB/c mice (Anlab Brno, Czech Republic) were used. Animals were housed individually in plastic cages and provided standard rodent chow and water *ad libitum*.

All studies described within this report are based on oocysts obtained from the intestinal content of 1 adult wild-caught *L. striatus* (R180/98). To obtain infectious material for further studies, *L. barbarus* ($N = 2$) were inoculated *per os* with 5000 oocysts suspended in water. The pre-patent and patent periods were established by examining the faecal samples every 12 h until the end of the experiment. Fresh faecal samples were collected and mixed with $K_2Cr_2O_7$, poured into Petri dishes to a depth < 5 mm, incubated at RT and repeatedly examined at 12 h intervals until a maximum percentage sporulation had been achieved.

Infections derived from 1 oocyst per rodent were performed to exclude a possible co-occurrence of more than 1 eimerian species. Oocysts from previously infected *L. barbarus* were diluted in commercial food-agar (Vitana a.s., Czech Republic) and observed in a thin layer under the microscope. After the agar had gelled, 1 oocyst with surrounding agar was carefully picked out by an injection needle, diluted in water, and inoculated *per os* to *L. barbarus* ($N = 3$), treated as described above.

Light and electron microscopy

L. barbarus ($N = 5$), each fed 1000 oocysts, were killed 2, 3, 4, 5, and 7 days p.i. Additionally, BALB/c mice ($N = 5$) were each inoculated with 5000 oocysts and sacrificed 15 days p.i. Uninfected *L. barbarus* and BALB/c mice (one of each) were used as controls. The intestinal contents of all rodents were screened for the presence of oocysts, as noted above. For histological examination, the following tissues were collected and fixed in 10 % buffered formalin: abdominal wall, liver, spleen, lungs, heart, kidney, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon and rectum. Specimens were processed for histology as paraffin sections (6 μm) and stained with H & E, periodic acid-Schiff (PAS) or Giemsa's stains. All stages were measured with a calibrated ocular micrometer.

For transmission electron microscopy (TEM), the tissues containing developmental stages were fixed in 2·5 % glutaraldehyde in 0·1 M cacodylate buffer (pH 7·4) at 4 °C and post-fixed in 1 % osmium tetroxide in the same buffer. Specimens were washed, dehydrated in graded alcohols and embedded in Durcupan. Ultrathin sections (100 nm) were stained with uranyl acetate and lead citrate and examined with a JEOL 1010 transmission electron microscope. Samples for scanning electron microscopy (SEM) were fixed in 4 % (w/v) buffered paraformaldehyde at 4 °C, rinsed in distilled water, dehydrated in alcohol and acetone series, desiccated by critical-point drying in carbon dioxide, gold-coated, and examined with a JEOL JMS 6300 scanning electron microscope.

DNA extraction, PCR, sequencing

Total cell DNA from *E. telekii* was isolated as described previously (Jirků *et al*. 1995). SSU rRNA gene was PCR amplified with the oligonucleotides K11 (AAAGATTAAGCCATGCA) and K12 (CAAAGGGCAGGGACGTA), which anneal to the conserved 5′ and 3′-end regions. Conditions were as follows: initial denaturation 95 °C for 4 min followed by 30 cycles at 95 °C for 1 min, 40 °C for 1 min, 72 °C for 1·5 min and a final extension at 72 °C for 10 min. Amplicon was purified on 0·75 % agarose gels, gel-isolated and cloned using TOPO™ TA Cloning version E (Invitrogen). Both strands were sequenced on an automated DNA sequencer using BigDye DNA Sequencing Kit (Perkin-Elmer).

Phylogenetic analysis

The alignment was generated using Clustal W with default parameters (Thompson, Higgins & Gibson, 1994), controlled by eye in order to eliminate ambiguously aligned nucleotides. It contained the SSU rRNA sequences of selected coccidian species and *E. telekii* (Table 1), and *Toxoplasma gondii* used as an outgroup. Since for some species, the complete sequence was not available due to missing regions at the 3′ and/or 5′ ends, the 183 nt at the 3′ end and

Table 1. Species list of coccidia indicating the hosts and accession numbers of the SSU rDNA

Species	Host	Accession number
*Toxoplasma gondii**	Cat vertebrates	M97703
Isospora robini	American robin	AF080612
Cyclospora cayetanensis	Human	AF111183
Cyclospora cercopitheci	African green monkey	AF111185
Cyclospora papionis	Olive baboon	AF111187
Eimeria acervulina	Domestic fowl	U67115
Eimeria brunetti	Domestic fowl	U67116
Eimeria maxima	Domestic fowl	U67117
Eimeria necatrix	Domestic fowl	U67119
Eimeria praecox	Domestic fowl	U67120
Eimeria mivati	Domestic fowl	U76748
Eimeria mitis	Domestic fowl	U40262
Eimeria tenella	Domestic fowl	U40264
Eimeria nieschulzi	Common rat	U40263
Eimeria falciformis	House mouse	AF080614
Eimeria telekii	Striped grass mouse	AF246717

* Used as outgroup.

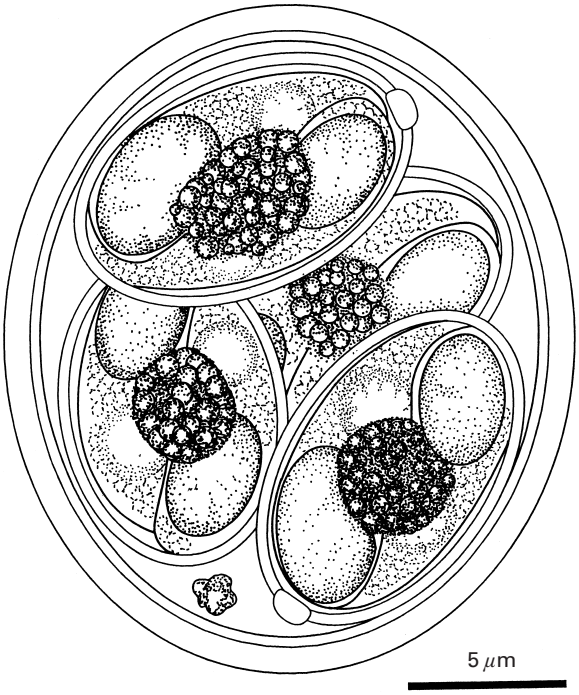

Fig. 1. Composite line drawing of sporulated oocyst *Eimeria telekii* n.sp.

55 nt at the 5′ end were removed from the alignment. Additionally 2 ambiguously aligned areas (26 nt) were removed from the alignment. Therefore, the analysis was based on 1561 nt-long region where sequence data were available for all the species under consideration. The sequence alignment in NEXUS format is available on request from J.R.Š. Phylogenetic relationships were reconstructed using the parsimony and maximum likelihood methods. Alignments were analysed by the program package PAUP* (Swofford, 1998). Parsimony analysis was performed using branch and bound search settings with gaps treated as missing data and transversion/transition ratio 1:1 and 1:3. Maximum likelihood was performed using the heuristic search settings and HKY 85 model of substitution. Bootstrap analysis (400 replicates for maximum likelihood, 1000 replicates for maximum parsimony) and the Bremer decay indices (number of extra steps for a clade not to be unequivocally supported) were performed.

RESULTS

Faecal pellets of 1/4 (25%) striped grass mice, *L. striatus*, captured in Chuka, Kenya, contained coccidian oocysts belonging to the genus *Eimeria*. Morphological and experimental results clearly suggest that the coccidium found represents a new species, the description of which is presented.

Oocyst morphology

Oocysts oval to spherical, occasionally ellipsoidal (Figs 1 and 2A, B). Oocyst wall colourless, smooth, bilayered, 1·3–1·5 thick; the outer layer is thicker, while the less prominent inner layer about 0·3–0·5 thick. Micropyle and oocyst residuum absent. Fine polar granule present, 1·0–1·5 in diameter. Oocysts 20·4 × 15·7 (15·5–25·0 × 12·0–20·0; $N = 50$), with the shape index 1·3 (1·17–1·58; $N = 50$). Sporulated oocysts contain 4 sporocysts, each with 2 sporozoites. Sporocysts ellipsoidal with a small and ellipsoidal Stieda body about 1·0 × 0·5 μm in size. A finely granular compact sporocyst residuum (2·0–3·0 in diameter) present. Sporocysts 11·2 × 7·8 (10·0–12·0 × 7·0–9·0; $N = 50$), the shape index 1·43 (1·33–1·57; $N = 50$). Sporozoites elongate and banana-shaped, finely granulated, each with 1 large oval refractile globule and barely visible nucleus.

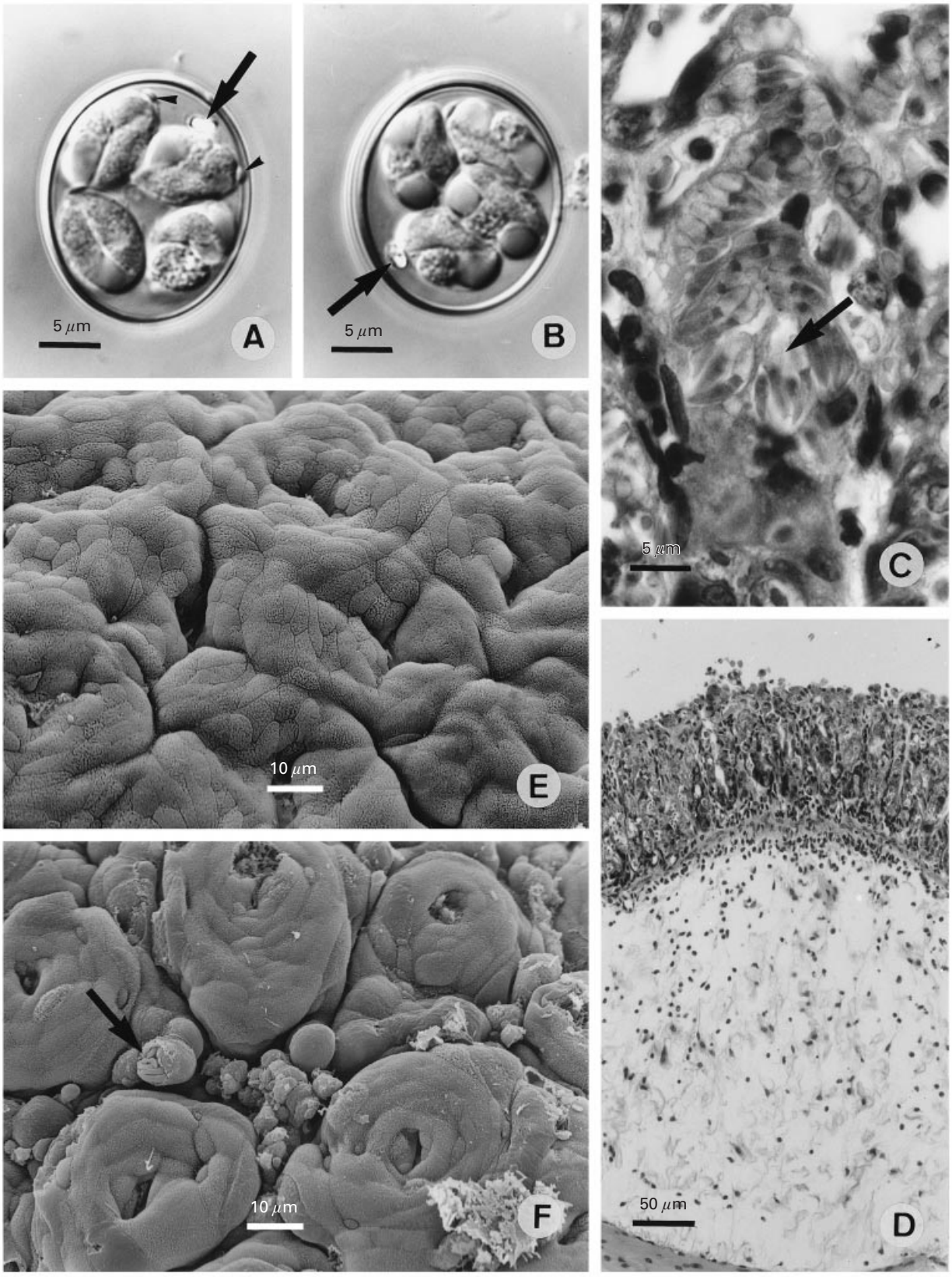

Fig. 2. (A–F) Oocysts and merogonial stages of *Eimeria telekii* n.sp. and pathological changes during the experimental infection of *Lemniscomys barbarus* with 1000 oocysts. (A, B) Nomarski interference contrast of sporulated oocyst of *E. telekii*; note polar granule (arrow) and Stieda body (arrowhead). (C, D) Histological section showing caecum (5th day p.i.) (H&E). (C) Numerous merogonial stages (arrow). (D) Histological section showing pathological lesions; note the mucosal acellular oedema. (E, F) SEM micrograph of the mucosal surface of caecum. (E) Absence of lesions at the 2nd day p.i. (F) Thickening, atrophy and necrosis of the infected epithelium with protruding enterocytes; ruptured enterocyte containing a meront filled with merozoites (arrow) at the 5th day p.i.

Morphological features of oocysts resulting from experimental 1-oocyst infections are identical with those obtained from the original Kenyan isolate.

Sporulation exogenous; first sporulated oocysts appearing 48 h post-defecation. After 98 h 86 % of oocysts sporulated and this number did not increase.

Pre-patent and patent periods

All 8 *L. barbarus* experimentally infected with 1 or 1000 oocysts began discharging oocysts on 4–5 days p.i. Oocyst discharge peaked during the 6th day p.i., and declined slowly in the following days. Oocysts were not detected in faeces of experimental animals after 12 days p.i.

Pathology and location in the host

Experimental infection of 2 *L. barbarus* with 5000 oocysts of *E. telekii* caused clinical coccidiosis. Infected animals became clinically ill 5–6 days p.i.; 1 died on the 6th day p.i., the other was euthanized being moribund on the 7th day p.i. The observed clinical signs comprised ataxia, weakness and slight diarrhoea with traces of blood in faeces. Experimental inoculation of *L. barbarus* with 1000 oocysts led to clinical signs of similar intensity and identical histological findings. At necropsy 5–7 days p.i. the caecum was dilated, filled with necrotic and haemorrhaged material, the caecal mucosa was hyperaemic and oedematous. The histological lesions observed in the caecum 5–7 days p.i. consisted of atrophy of the crypts associated with a large number of developmental stages of *E. telekii* inside the enterocytes, epithelial necrosis, oedema, haemorrhages and mixed inflammatory infiltrate (Fig. 2C, D). The colon was less affected. The l-oocyst infection caused only slight lethargy 5–8 days p.i. The wild *L. striatus* (= symbiotype) did not show any signs of clinical illness; although histological examination revealed developing oocysts measuring *in situ* $14{\cdot}6 \times 12{\cdot}9$ ($16–14 \times 11–$; $N = 15$) within the enterocytes of the colon and caecum.

SEM examination of the intestinal surface of the caecum of *L. barbarus* infected with 1000 oocysts revealed no pathological changes 2 days p.i. (Fig. 2E). However, 5 days p.i. numerous erosions were observed in the caecal mucosa, with the enterocytes being enlarged, ballooned and ruptured, releasing second-generation merozoites (Fig. 2F).

Merogony and gamogony

The endogenous stages of *E. telekii* were first seen in the colonic and caecal mucosa 2 days p.i. Only very few meronts found, $18{\cdot}4 \times 13{\cdot}2$ ($17–19{\cdot}0 \times 12–15{\cdot}0$; $N = 5$), contained 21 (16–24; $N = 5$) elongate slightly tip-sharpened first-generation merozoites, $1{\cdot}2 \times 8{\cdot}0$ ($1–1{\cdot}5 \times 6–9{\cdot}0$; $N = 10$), having prominent nuclei.

At day 3 p.i., the immature second-generation meront was at 52 % ($N = 150$) the prevailing developmental stage. Mature meronts contained elongate, banana-shaped mature merozoites with centrally located nucleus and rounded and pointed ends, the latter being more basophilic. The tips of the merozoites were not as sharpened as those of the first-generation merozoites. The PAS staining revealed several PAS-positive granules inside the merozoites close to the rounded end at days 3–5 p.i.

At day 4 p.i., the mature second-generation meront was at 40·7 % ($N = 150$) the prevailing developmental stage. The mature meronts, $14{\cdot}2 \times 10{\cdot}2$ ($10–18{\cdot}0 \times 7–12{\cdot}0$; $N = 50$), contained 11 (5–15, $N = 50$) merozoites, $2{\cdot}3 \times 11{\cdot}8$ ($2–3{\cdot}0 \times 10–15{\cdot}0$; $N = 50$). Mature microgamonts, $9{\cdot}5 \times 8{\cdot}0$ ($8–12{\cdot}0 \times 5–10{\cdot}0$; $N = 10$) in diameter, contained approximately 50–100 microgametes $1{\cdot}0 \times 2–2{\cdot}5$ in size. Immature macrogamonts each with a distinct nucleus and nucleolus measured $13{\cdot}5 \times 11{\cdot}0$ ($12–16{\cdot}0 \times 10–14{\cdot}0$; $N = 30$) while mature macrogamonts with marginally arranged granules were $14{\cdot}5 \times 13{\cdot}0$ ($13–17{\cdot}0 \times 12–15{\cdot}0$; $N = 30$) in size. Developing oocysts observed very rarely (1·3 %).

At day 5 p.i., sexual stages and oocysts prevailed. Developing oocysts, $15{\cdot}4 \times 12{\cdot}5$ ($14–17{\cdot}0 \times 10–14{\cdot}0$; $N = 50$), contained an eosinophilic sporont and were distinguished from mature macrogametes by the preformed oocyst wall. At day 7 p.i., oocysts prevailing ($N = 150$). Only very few remaining second-generation meronts seen at this stage of infection.

The analysis of second-generation merozoites in TEM revealed typical coccidian features. Merogony (ectomerogony) takes place within the parasitophorous vacuole inside the enterocyte (Fig. 3A). Electron-dense droplets, amylopectin-like granules and mitochondria also occur in the cytoplasm of mature merozoite (Fig. 3B, C). As seen in Fig. 3D, some enterocytes appeared to be parasitized by more than 1 meront.

Host specificity

While *L. barbarus* is a susceptible host, BALB/c mice did not show any sign of clinical changes and none of them passed oocysts in their faeces up to 15 days p.i. No endogenous stages were seen during the histological examination of tissue samples from mouse killed 5 days p.i.

Phylogenetic position

The parsimony analysis yielded a single most parsimonious tree of 379 steps (consistency index 0·7467; retention index 0·6811) (Fig. 4). *Cyclospora* spp. of primates formed a sister group of *Eimeria* spp. from the domestic fowl. Since the rodent eimeriids (*E. nieschulzi*, *E. falciformis*, and *E. telekii*) and *Isospora robini* constituted an early branching

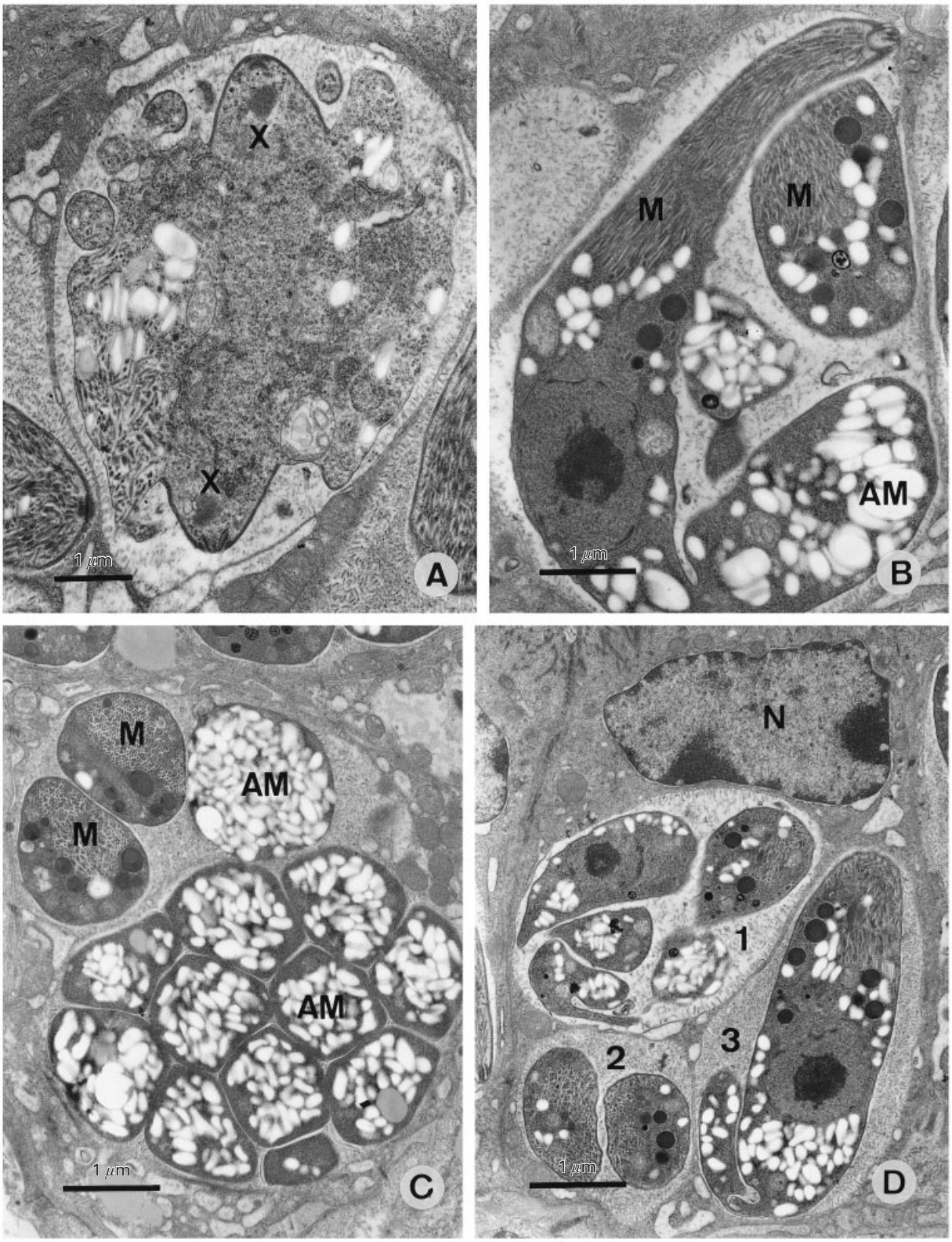

Fig. 3. (A–D) TEM of the merogonial stages in enterocytes of *Lemniscomys barbarus* experimentally infected with 1000 oocysts of *Eimeria telekii* n.sp. (3rd day p.i.). (A) Developing meront inside the enterocyte; note the parasitophorous vacuole and developing merozoites by ectomerogony and formation of the apical complex (X). (B) Mature merozoite with centrally located nucleus and distinct nucleolus; the apical part is filled with micronemes (M), and the opposite end contains numerous amylopectin-like granules (AM); (C) Cross-section through a mature meront showing large number of merozoites filled with amylopectin-like granules (AM) and micronemes (M). (D) Section through an enterocyte infected with 3 meronts (1, 2, 3), each within its own parasitophorous vacuole.

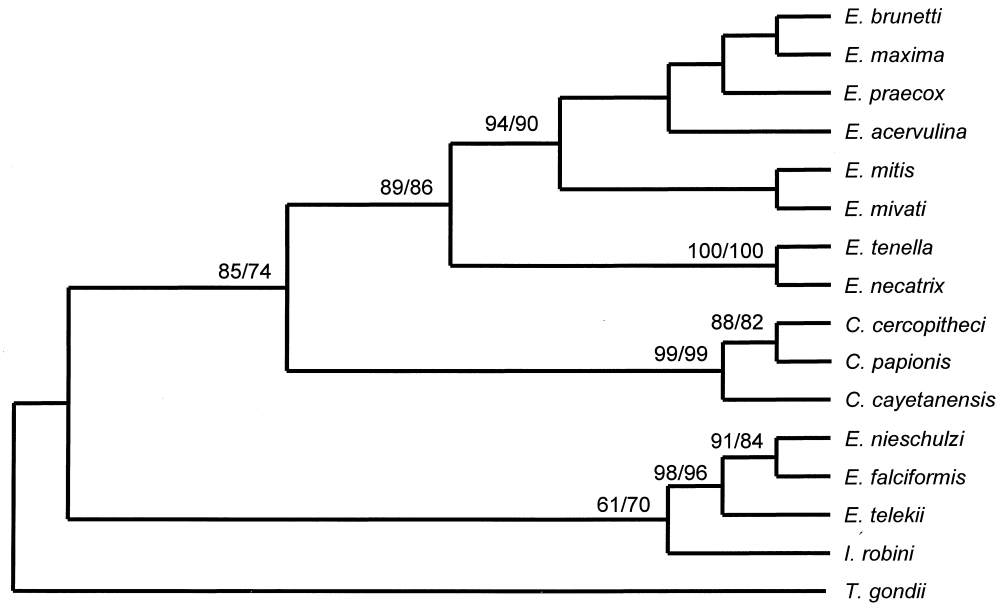

Fig. 4. Parsimony tree of the SSU rRNA genes (length = 379; consistency index = 0·7467; retention index = 0·6811). Numbers on branches indicate the bootstrap support for maximum parsimony/maximum likelihood using the heuristic algorithm generated in PAUP* (Swofford, 1998). The tree is rooted at *T. gondii*.

monophyletic group that is a sister group to *Cyclospora* spp. and all other *Eimeria* spp., the genus *Eimeria* appeared paraphyletic in our dataset. The bootstrap support is high for monophyly of the rodent clade (Fig. 4). Furthermore, the Bremer index underlined its robustness since the entire tree collapsed into an overall polytomy after 8 additional steps, while the rodent species clade resisted 7 additional steps.

The tree obtained using maximum likelihood (data not shown) had a ln likelihood of −4429·51834. This tree was identical with the one constructed by parsimony (Fig. 4), the only difference being an exchange between the positions of *E. brunetti* and *E. praecox*.

DISCUSSION

There are 2 reports of *Eimeria* in *L. striatus* and our isolate differs from them in several aspects. Bray (1958) and Levine *et al.* (1959) described *E. putevelata* and *E. lemniscomysis*, respectively, which can easily be distinguished by smaller oocysts, shape of sporocysts, site of infection, and other features. Similarly, other eimerian species described from murid rodents of sub-Saharan Africa differ significantly from the species described here by the oocyst morphology and were found in hosts phylogenetically distant from *Lemniscomys* (De Vos & Dobson, 1970; Levine *et al.* 1959). The conspecificity of our coccidium with some *Eimeria* species parasitizing domestic mice *Mus musculus* was excluded by negative results of the cross-transmission experiment.

Many *Eimeria* species usually develop 2 asexual merogonous generations before starting the sexual phase of the life-cycle (Long, 1982). Indeed, during the endogenous development of *E. telekii*, 2 asexual generations were observed proceeding the sexual generation. Pathological lesions described above are similar to symptoms associated with other rodent eimerian infections, e.g. *E. straconicensis* from *Microtus arvalis* (Koudela & Vítovec, 1994) and *E. pragensis* affecting *Mus musculus* (Mesfin, Bellamy & Stockdale, 1978). The caecum and colon are the preferred sites of infection by *E. telekii*. The first generation meronts containing large numbers of merozoites appeared very quickly after the inoculation but did not cause significant pathological changes. Using SEM, the inner surface of the caecum seemed to be intact until 2 days p.i. However, severe pathological lesions were induced by the second merogonial generation and sexual stages. The acellular oedema observed on the 5th day p.i. was similar to the oedema reported in the colon of mice infected by *E. pragensis* in the same phase of infection (Mesfin *et al.* 1978). The overall pathological changes were comparable to those reported for intestinal coccidioses of other vertebrates (Fernando, 1982, 1990; Gregory, 1990).

An increasing number of oocysts ingested by the host is usually accompanied by increasing severity of the disease (Fernando, 1982; Gregory, 1990; Norton, Catchpole & Joyner, 1979). Our findings further confirm this correlation. While mice infected with 1 oocyst did not show any signs of illness, those receiving 1000 oocysts exhibited severe clinical and pathological changes but survived the infection. The inoculum of 5000 oocysts was lethal for experimental hosts. The minimum threshold dose above which death occurs is within 1000 and 5000 oocysts inoculated.

Table 2. Comparison of morphology and biology of up to date known *Eimeria* species from striped grass mice of the genus *Lemniscomys**

	E. telekii n.sp.	*E. putevelata*	*E. lemniscomysis*
Oocyst	20·4 × 15·7 (15·5–25·0 × 12·0–20·0) Oval to spherical	25·6 × 19·9 (30–22 × 22–17) Oval	28·3 × 18·8 (27–30 × 18–19) Broadly spindle-shaped with somewhat flattened ends
Shape index	1·3 (1·17–1·58)	N.A.	1·5–1·6
Sporocyst	11·2 × 7·8 (10·0–12·0 × 7·0–9·0) Ellipsoidal	11·5 × 8·3 (13–10 × 10–8) Ovoid	16 × 8 Elongate ovoid, pointed at one end
Sporozoite	Elongate, banana-shaped	10 × 3 Banana-shaped	N.A.
Oocyst wall	Bilayered, smooth and colourless, outer layer was thicker, < 1 and the inner layer was *ca* 0·3–0·5	Bilayered, outer thick and yellow, covered with small well-defined pits	Single-layered, brownish yellow, moderately rough and pitted, *ca* 1·2 thick at the sides and 0·8 at the ends (thin membrane lines the wall)
Micropyle	No	No	No
Oocyst residuum	No	No	No
Polar granule	Present 1–1·5	N.A.	Present
Stieda body	Small and ellipsoidal 1 × 0·5	Very small, only slight thickening	Small or absent
Sporocyst residuum	Present (finely granular)	Usually but not always	Present (finely granular)
Sporulation	2·5–3·5 days	10 days	3 days
Site of infection	Caecum, colon	Ileum	Upper jejunum and lower duodenum
Host	1 of 4 *L. striatus*; experimentally *L. barbarus*	1 of 1 *L. striatus*	1 of 11 *L. striatus*
Type locality	Kenya	Liberia	Liberia
Reference	This study	Bray (1958)	Levine *et al.* (1959)

* Measurements are in μm as the mean followed in parentheses by the range.
N.A.; Not available.

PAS-positive granules inside eimerian sporozoites and merozoites are known to contain amylopectin; individual species and also developmental stages differ from each other in the amount of these granules (Ball & Pittilo, 1990; Scholtyseck, 1979). Amylopectin is thought to be a form of polysaccharide storage of *Eimeria*. It was shown that amylopectin of *E. tenella* plays an important role in establishing the infection (Nakai & Ogimoto, 1987). First-generation merozoites of *E. bovis* consist of 2 types of merozoites differing in size, shape and amount of amylopectin granules and micronemes, each suggested to possess a unique role in the life-cycle. The merozoites with high amounts of amylopectin are highly motile and capable of penetrating cultured cells (Speer, 1988). Second-generation merozoites of *E. telekii* contain large numbers of PAS-positive granules that were identified by TEM as amylopectin-like electron-lucent granules. Only one type of second-generation merozoites with a large amount of amylopectin granules at the caudal end was observed in *E. telekii* infections. In other aspects the cellular organization of merogonic stages of *E. telekii* was similar to that observed in other eimerian coccidia affecting mammalian hosts (Ball & Pittilo, 1990; Scholtyseck, 1979).

Generally, eimerian parasites are regarded to be genus specific (Kogut, 1990). Duszynski (1986) questioned host specificity of the rodent *Eimeria* spp., and in a review Levine & Ivens (1988) showed that in rodent to rodent transmission experiments 80% and 12·5% of congeneric and intergeneric transmissions, respectively, were successful. Our isolate of *E. telekii* from *L. striatus* was able to infect the congeneric host *L. barbarus* while experimental transmission to laboratory mice failed. Thus, it seems possible that *E. telekii* may parasitize other species of the genus *Lemniscomys* in sub-Saharan Africa, since the ranges of distribution of *Lemniscomys* species overlap widely. However, *L. barbarus*

generally live in more arid habitats and strictly syntopic occurrence of *L. striatus* and *L. barbarus* was not reported (Nowak, 1991). The pathogenicity of *E. telekii* for *L. striatus* was not experimentally studied and needs to be proved also in this natural host.

The oocyst morphology is considered to be the most important morphological criterion in the eimerian taxonomy. Morphological variation of sporulated oocysts within individual eimerian species has been reported repeatedly (Duszynski, 1971; Gardner & Duszynski, 1990; Dyszynski, *et al*. 1992). Moreover, in some cases not only the variation in oocyst and sporocyst size, but also variation of the shape index, oocyst wall texture and morphology of sporocyst residuum and polar bodies exist. The problem of co-occurrence of more species in wild isolates of coccidia has been discussed previously (Parker & Duszynski, 1986). Although *E. telekii* is polymorphic in the size and shape of oocysts, experimental infections derived from 1 oocyst excluded possible presence of more species of *Eimeria* in the original material.

In the review of the molecular phylogeny of Apicomplexa, Ellis, Morrison & Jeffries (1998) clearly demonstrated the usefulness of the SSU rRNA gene for phylogenetic analysis of this protozoan phylum. For *Eimeria* species attention has been focused particularly on the phylogeny of domestic avian species (Barta *et al*. 1997), Stieda bodied-avian *Isospora* species (Carreno & Barta, 1999), and the relation between the genera *Eimeria* and *Cyclospora* (Lopez *et al*. 1999). Recently, the phylogenetic analysis of rodent *Eimeria* spp. has been extended to the internal spacer-1 of the rRNA region (Hnida & Duszynski, 1999*a*). In the SSU rRNA-based analysis *E. telekii* appeared in a clade with *E. nieschulzi* and *E. falciformis* that are the only other rodent species for which the SSU rRNA sequence is available. Such a close relatedness of rodent eimeriids originating from different continents is remarkable and testifies for a certain level of coevolution of *Eimeria* spp. with their hosts. Similar coevolution was noticed for another coccidian genus *Sarcocystis* (Tenter, Baverstock & Johnson, 1992; Doležel *et al*. 1999). The stability of the rodent clade was tested by the Bremer index and bootstrap analysis and proved to be very stable. The branching order of analysed trees was not affected by the use as outgroups of distantly related *Cryptosporidium parvum*, *Toxoplasma gondii* and/or more closely related *Caryospora bigenetica* (data not shown).

Parasitological biodiversity and information from studies of parasites help to understand the biosphere from the global point of view (Brooks & Hoberg, 2000). To our knowledge *E. telekii* represents the first eimerian isolate from the African continent that has been studied in detail.

TAXONOMIC SUMMARY

***Eimeria telekii* n.sp.**

Type-host: *Lemniscomys striatus* (Linnaeus, 1758) (striped grass mouse).

Type-locality: Chuka, Mt Kenya region, Kenya (approximately 00° 21′ S; 37° 42′ E); altitude 1550 m.

Sporulation: Exogenous.

Site of infection: Caecum and colon.

Prevalence: 25% (1/4) *L. striatus* infected.

Type-specimens: Phototypes are deposited at the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, No. R180/98. Symbiotype (ethanol preserved) is deposited at the ZFMK (No. 99561).

Etymology: The specific epithet '*telekii*' is derived as genitive of the name of Count Teleki von Szek, who was one of the early explorers in the Eastern Africa to report about Mt Kenya in 1883.

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